

MANGROVE ECOSYSTEMS

**A MANUAL FOR THE ASSESSMENT
OF BIODIVERSITY**

**A follow up of the
National Agricultural Technology Project
(NATP.), ICAR.**

*Mangrove Ecosystem Biodiversity :
Its Influence on the Natural Recruitment of
Selected Commercially Important Finfish and Shellfish
Species in Fisheries*

Edited by :
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P.B. No. 1603, Ernakulam North P.O; Cochin – 682 018, Kerala, India







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A Manual for the Assessment of Biodiversity

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Macrobenthos - Methods for Study

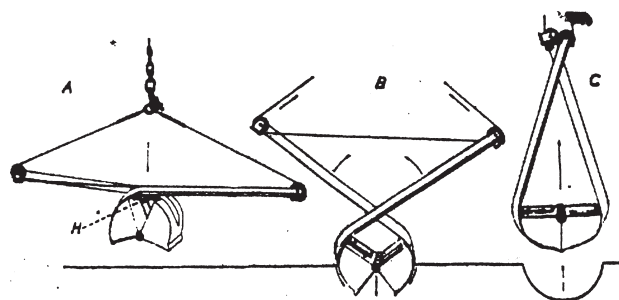
K. Vijayakumaran

The bottom fauna or benthos forms an important link in the food web of the aquatic ecosystems. Bottom dwelling fishes and crustaceans feed mainly on the benthic organisms and hence the abundance of benthic fauna is a major factor deciding the fishery potential of watery body. In recent years, long-term works on benthic communities has been gaining importance in pollution studies and assessment of ecosystem health. Therefore, study of the qualitative and quantitative aspects of benthos is very important in mangrove ecosystem biodiversity determination.

The macrofauna and meiofauna are mainly distinguished by their relative size and groups involved. It is generally accepted that those animals retained in 0.5 – 2.0 mm mesh depending on the nature of the substratum sieve are macrofauna and those which pass through these sieves but are retained by a sieve of about 60 μ m mesh are termed meiofauna.

Sampling

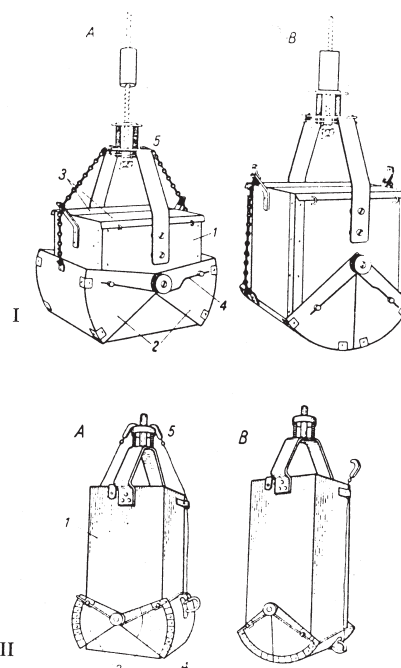
A Peterson grab or van Veen grab (Fig.1) or Eckman grab can be conveniently used for collecting benthos samples. Surface area of grab is recorded and number & biomass are expressed in cm^2 with the



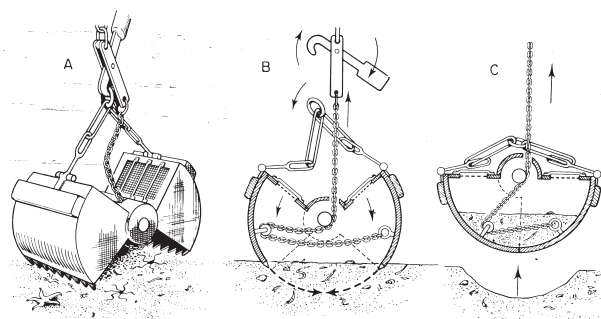
van Veen grab in different stages of operation

required conversion / multiplication for expressing number and biomass. While taking the grab sample quantity of the sediment must be checked because improper performance of grab may result in reduced quantity of sediment and thus erroneous values of population and biomass. In case of improper functioning of the grab, the sampling operation must

be repeated. Record the sediment temperature immediately after the grab is taken out. Remove a sub-sample if sediment characteristics are to be analyzed.



Ekman - Birge grab. I. Initial form; II. Tall form as used by Borutzky. A, Open; B, closed. It is advantageous to use flexible cable rather than chains to hold the jaws open and to have the guides open rather than to have the cable pass through a hole. 1, box; 2, jaws; 3, top lids; 4, spring which operates jaws; 5, jaw chain fastened on trip mechanism.



Petersen grab taking a sample on the sea bed (Redrawn from Hardy, 1959, and reproduced with permission from *Advances in Marine Biology*, Vol. 2)

Processing of sample

Transfer the sediment without spilling into a tub or bucket. Wash the sediment through a sieve with copious flow of water, taking care not to spill over or clog the mesh. A series of sieves can be used if the size composition of the fauna has to be determined. Three sieves of different mesh sizes 2000 μm (BSS-8), 1000 μm (BSS-16) and 500 μm (BSS-30) in that order would give reasonably good information about the size structure of the fauna (Vijayakumaran, 2003). The different specification (numbers) of test sieves and the corresponding mesh sizes are given in *Appendix -3*. If information on size is not important a single sieve of 500 μm can be used for segregating the macrofauna.

The benthic organisms retained in each sieve have to be collected in separate bottles and preserved with 4 percent formalin onboard (collect the sample in approximately 100 ml water and add 10 ml of 40% formalin). A few drops of Rose Bengal stain (1:500) added to this sample would facilitate sorting as the organisms would take deep purple colour. Use of a soft brush for transferring animals is quite desirable.

Processing in the Laboratory

Larger animals can be picked up, enumerated and weighed separately before segregation and enumeration of smaller animals is done. Smaller animals can be sorted and enumerated under a dissection microscope using fine brushes, needle and forceps. Care must be taken not to exert pressure on any delicate worms to cause breakage and counting of the two pieces as separate animals. The displacement volume and wet weight (after properly blotting the moisture with filter paper) of animals sorted out can be taken before identification and enumeration. The organisms may be kept on filter paper and adhering moisture can be removed without damaging the animal and wet weight may be taken by a precession balance. After taking the weight the qualitative composition of sample is ascertained.

Before estimation of dry weight, shells and other hard materials should be removed. The organisms can be dried at 80 °C to constant weight. (if necessary it can also be dried at 105 °C and weigh without much delay, but fat content may be melted out which may

affect the exact weight). Being a destructive process, it is not always practicable to estimate the dry weight of all samples. A convenient method will be to workout a formula for conversion of wet weight (or volume) into dry weight. This can be achieved by taking dry weight of a few numbers of samples of which wet weight (volume) are known and working out a conversion factor based on the relationship.

The sorted animals can be preserved in alcohol in glass specimen tubes (some plastic tubes such as Tarson's tubes may crack or leak and cause drying up of specimens). Formalin should never be used for preserving or fixing Sponges and Ctenophores, since rapid maceration or total destruction of the animal may occur. Though formalin is a better fixative than alcohol in microscopy, other reagents such as Formaldehyde Alcohol Acetic acid (FAA) or Bouin's reagent are preferable. A more convenient preservative is the alcohol-glycerin, in which glycerin will prevent total desiccation as well as act as a clearing agent (see *Appendix-2* for methods of preparation)

A systematic order has to be followed in recording the number of animals under different taxa. A tentative listing is provided in *Appendix-1*. Organisms difficult to identify must be kept in separate specimen tubes properly labeled for future identification. After proper identification, the same can be incorporated in the data sheet of the relevant sample. The different ecological indices can be worked out as per the formulae given in *Appendix-4*.

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Appendix – 1**SUGGESTED CLASSIFICATION OF FAUNA**

A typical systematic order of benthic and planktonic organisms is presented below as per UNESCO (1983) and Gosner (1971). The changes that may come into effect in the classification can be incorporated without affecting the general pattern.

PROTOZOA

FORAMINIFERA

RADIOLARIA

PORIFERA

CALCAREA

HEXACTINELLIDA

DEMOSPONGIA

CNIDARIA

HYDROZOA

SCYPHOZOA

ANTHOZOA

CTENOPHORA**PLATYHELMINTHES**

TURBELLARIA

TREMATODA

CESTODA

RHYNCHOCOELA**ASCHELMINTHES**

ROTIFERA

GASTROTRICHA

KINORHYNCHA

PARIPULIDA

NEMATODA

NEMATOMORPHA

ENTOPROCTA**ECTOPROCTA****TARDIGRADA****CHAETOGNATHA****BRYOZOA****PHORONIDA****BRACHIOPODA****MOLLUSCA**

POLYPLACOPHORA

APLACOPHORA

GASTROPODA

SCAPHOPODA

BIVALVIA

CEPHALOPODA

ANNELIDA

POLYCHAETA

MYZOSTOMARIA

OLIGOCHAETA

HIRUDINEA

SIPUNCULIDA
 ECHIURIDA
 ARTHROPODA

MEROSTOMATA
 ARACHNIDA
 PANTOPODA
 CRUSTACEA

Cephalocarida
 Branchiopoda
 Ostracoda
 Copepoda
 Mystacocarida
 Branchiura
 Cirripedia
 Malacostraca

INSECTA

PYCNOGONIDA
 POGONOPHORA
 ECHINODERMATA

CRINOIDEA
 HOLOTHUROIDEA
 ECHINOIDEA
 ASTEROIDEA
 OPHIUROIDEA

HEMICHORDATA
 TUNICATA
 CEPHALOCHORDATA
 CYCLOSTOMATA
 SELACHII
 TELEOSTEI

Appendix- 2

Lugol's Iodine

Dissolve 100 g KI in 1 litre of distilled water then dissolve 50-g iodine (crystalline) and add 100 ml of glacial acetic acid. Decant the solution to remove precipitates.

Bouin's Reagent

Mix 75 parts picric acid (saturated solution made by dissolving 1 g of picric acid crystals to about 75 ml distilled water), 25 parts formalin and 5 parts glacial acetic acid.

FAA

Mix 10 parts formalin, 50 parts 95 % alcohol, 2 parts glacial acetic acid 40 parts water.

Alcohol-Glycerin

Mix 19 parts 70% alcohol (ethyl or isopropyl) and 1 part glycerin.

Appendix - 3**SPECIFICATIONS OF TEST SIEVES**

*B.S.S(410/1969)	A.S.T.M.(11-70)	I.S.I(460/1972)	Mesh size(μ m)
4	5	4.00 mm	4000
5	6	3.35 mm	3353
6	7	2.80 mm	2812
7	8	2.36 mm	2411
8	10	2.00 mm	2057
10	12	1.70 mm	1680
12	14	1.40 mm	1405
14	16	1.18 mm	1204
16	18	1.00 mm	1003
18	20	850 μ m	850
22	25	710 μ m	710
25	30	600 μ m	600
30	35	500 μ m	500
36	40	425 μ m	420
44	45	355 μ m	355
52	50	300 μ m	300
60	60	250 μ m	250
72	70	212 μ m	210
85	80	180 μ m	180
100	100	150 μ m	150
120	120	125 μ m	120
150	140	106 μ m	105
170	170	90 μ m	90
200	200	75 μ m	75
240	230	63 μ m	63
300	270	53 μ m	53
350	325	45 μ m	45
400	400	37 μ m	37
500	-	25 μ m	25

* B.S.S. = British Standard Sieves; ASTM = American Standards Test Mesh; ISI = Indian Standards Institution

Appendix – 4

ECOLOGICAL INDICES

INDICES OF DISPERSION

A number of indices based on variance to mean ratio (as the variance and mean are equal in theoretical Poisson distribution) has been suggested to test i) the *equality* of the variance-to-mean in a Poisson series and ii) measure the degree of clumping of a population of organisms. The details of formula of three such ratios being widely used are given below.

Index of Dispersion (ID)

This, being the variance to mean ratio, is the simplest of all indices of dispersion and is calculated as:

$$ID = \frac{s^2}{\bar{x}}$$

where \bar{x} and s^2 are sample mean and variance respectively.

Index of Clumping (IC)

A modification of Index of Dispersion suggested by David and Moore (1954) is termed as Index of Clumping (IC) given by the formula:

$$IC = (s^2 / \bar{x}) - 1 = ID - 1$$

where \bar{x} and s^2 are sample mean and variance respectively.

Green's Index (GI)

When population is clumped, ID is strongly influenced by 'n' the number of individuals in the sample. Green (1966) suggested a modification to Index of Clumping, which is independent of 'n' and is known as Green's Index (GI)

$$GI = \frac{(s^2 / \bar{x}) - 1}{n - 1} = \frac{IC}{n - 1}$$

The properties of the three indices of dispersion at maximum regularity, randomness and maximum clumping are summarized in the following table:

Morisita's Index (I_d)

Morisita (1971) proposed an index, (almost similar to the Lloyd's Index of Patchiness), that is unaffected by changes in density due to random thinning. Morisita's index is calculated as:

$$I_d = \left(\frac{n}{n-1} \right) \left(\frac{\bar{x}^*}{\bar{x}} \right)$$

Where, n is the total number of individuals in the sample and \bar{x}^* is the mean crowding given by the formula:

$$\bar{x}^* = \bar{x} + IC$$

DIVERSITY INDICES

The concept of species diversity in community ecology has been intensely debated by the ecologists over the years. Species diversity may be thought of as being composed of two components. The first is the number of species in the community, which ecologists often refer to as species **richness**. The second component is the species **evenness** or **equitability**.

There are literally an infinite number of diversity indices. Two of the commonly used indices, which are also needed for Hill's Diversity numbers, are Simpson's index and Shannon's Index.

Simpson's Index (λ):

Simpson (1949) proposed the first diversity index used in ecology as:

$$\lambda = \sum_{i=1}^s p_i^2$$

Where p_i is the proportional abundance of the i th species, given by

$$p_i = \frac{n_i}{N}, i = 1, 2, 3, \dots, S$$

INDEX	VALUE OF INDEX AT		
	Maximum Uniformity	Complete Randomness	Maximum Clumping
Index of Dispersion(ID)	0	1	n
Index of Clumping (IC)	- 1	0	n - 1
Green's Index (GI)	- 1/ (n-1)	0	1

and n_i is the number of individuals of the i^{th} species and N is the known total number of individuals for all S species in the population.

Shannon's Index H'

Shannon index H' has been probably the most widely used index in community ecology. Two of the salient features of this index are that H' assumes value 0 if only one species is observed in the sample and H' assumes maximum value when all S species are represented by the same number of individuals.

$$H' = \sum_{i=1}^S \left(\frac{n_i}{n} \right) \ln \left(\frac{n_i}{n} \right)$$

Where, n_i is the number of individuals belonging to the i^{th} of S species in the sample and n is the total number of individuals in the sample

The series of diversity numbers presented by Hill (1973) are probably the easiest to interpret ecologically.

Hill's Diversity Number 0:

$$N0 = S$$

Where S is the total number of species. $N0$ is obviously the number of all species in the sample regardless of their abundance.

Hill's Diversity Number 1:

$$N1 = e^{H'}$$

Where H' is Shannon's index defined above. $N1$ gives the number of abundant species in the sample.

Hill's Diversity Number 2:

$$N2 = 1/\lambda$$

Where λ is Simpson's index defined above. $N2$ gives the number of very abundant species in the sample.

Richness Indices

A straight forward index of species richness would be S , the total number of species in a community. However since S depends on the sample size, its utility as a comparative index is limited. Therefore a number of indices have been suggested based on the relation between S and n , the total number of individuals observed, which increases with increasing sample size. The two well-known richness indices are given below:

Margalef (1958) Index

$$R1 = \frac{S-1}{\ln(n)}$$

Where S is the total number of species in the community and n is the total number of individuals observed.

Menhinick (1964) Index

$$R2 = \frac{S}{\sqrt{n}}$$

Where S is the total number of species in the community and n is the total number of individuals observed.

Evenness Indices

In an attempt to quantify the evenness component of diversity, a number of indices have been proposed. Five of the commonly used evenness indices are described below:

(H , S , $N0$, $N1$, $N2$ and λ are as defined earlier)

Evenness Index 1 (E1).

This is the familiar J' of Pielou (1975,1977) and is probably the most common evenness index used by ecologists

$$E1 = \frac{H'}{\ln S} = \frac{\ln(N1)}{\ln(N0)}$$

Evenness Index 2 (E2).

Sheldon (1969) proposed an exponentiated form of $E1$ as an evenness index, which is calculated as:

$$E2 = \frac{e^{H'}}{S} = \frac{N1}{N0}$$

Evenness Index 3 (E3).

Heip (1974) proposed an index from which the minimum of the diversity index is subtracted and is expressed as:

$$E3 = \frac{e^{H'} - 1}{S - 1} = \frac{N1 - 1}{N0 - 1}$$

Evenness Index 4 (E4).

Hill (1973 a&b) proposed the ratio of $N2$ to $N1$ (ratio of number of very abundant species to abundant species) as evenness index, which will tend to become one as a single species tend to dominate.

$$E4 = \frac{1/\lambda}{e^{H'}} = \frac{N2}{N1}$$

Evenness Index 5 (E5).

This index, similar in form to $E3$ and known as the modified Hill's ratio, approaches zero as a single species becomes more and more dominant in a community. This is a desirable property for an evenness index:

$$E5 = \frac{1/\lambda - 1}{e^{H'} - 1} = \frac{N2 - 1}{N1 - 1}$$

Suggested Reading

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